

What is claimed is:

1. A disposable solid test strip capable of enabling a person to self-monitor fat loss on a daily basis in a fluid sample of urine, saliva, or sweat or other bodily fluid by providing a color signal, a photochemical signal or an electrochemical signal indicative of at least the  $\beta$ -hydroxybutyrate content of the sample upon being dipped in said sample, removed, allowed to rest briefly and then read.
2. A disposable solid test strip according to Claim 1 wherein the color, photochemical signal or electrochemical signal is indicative of the combined  $\beta$ -hydroxybutyrate and acetoacetate content of the sample.
3. A disposable solid test strip according to Claim 1 wherein the color, photochemical signal or electrochemical signal is indicative of the content of total ketone bodies present in the sample.
4. A solid test strip according to Claim 1 which comprises
  - 1) an inert support layer and
  - 2) a dried reagent layer comprising a porous material impregnated with
    - a)  $\beta$ -hydroxybutyrate dehydrogenase enzyme (" $\beta$  - HBD")
    - b) nicotinamide adenine dinucleotide ("NAD"),
    - c) a tetrazolium dye precursor

- d) an electron mediator capable of transferring an electron to said dye precursor to effect a color change and
  - e) a sufficient quantity of a buffer having a pH of from about 8.6 to about 9.5 to maintain the reaction pH at a level between about 8.6 and about 9.5 when the strip is saturated with a sample of bodily fluid.
5. A solid test strip according to Claim 4 in which the  $\beta$ -HBD enzyme is obtained from *Alcaligenes* or another source which contains  $\beta$ -HBD that is not inhibited by chloride ions and is present in an amount of from about 0.2 to about 5.0 U per strip.
6. A solid test strip according to Claim 4 wherein the tetrazolium dye precursor is nitrobluetetrazolium ("NBT") or 2-(indophenyl)-3-(paranitrophenyl)-5-phenyl tetrazolium chloride ("INT").
7. A solid test strip according to Claim 4 wherein the  $\beta$ -hydroxybutyrate is from a source that is inhibited by chloride ions and is present in an amount per strip from about 1 to about 100 U per strip.
8. A solid test strip according to Claim 4 wherein the electron mediator is a diaphorase enzyme.

9. A test strip according to Claim 2 which is comprised of
- 1) a inert support layer, and
  - 2) a dried reagent layer comprising a porous material impregnated with:
    - a)  $\beta$ -HBD enzyme
    - b) NAD
    - c) a tetrazolium dye precursor,
    - d) an electron mediator capable of transferring an electron to said dye precursor to effect a color change and
    - e) a sufficient quantity of a buffer having a pH between about 7.0 and about 8.3 to maintain the reaction pH between about 7.0 and about 8.3 when the strip is saturated with sample.
10. A test strip according to claim 9 wherein the  $\beta$ -HBD is obtained from *Alcaligenes* or another source found to produce  $\beta$ -HBD that is uninhibited by chloride ions and is present in an amount of from about 0.2 to about 5.0 U per strip.
11. A test strip according to claim 9 wherein the  $\beta$ -HBD is obtained from a source such that it is inhibited by chloride ions, and it is present in an amount per strip from about 1 to about 100 U per strip.
12. A test strip according to Claim 9 wherein the tetrazolium dye precursor is NBT or INT.

13. A test strip according to Claim 9 wherein the electron mediator is a diaphorase enzyme.
14. A test strip according to Claim 2 comprising:
  - 1) an inert support layer and
  - 2) a dried reagent layer comprising a porous material impregnated with:
    - a) NAD,
    - b)  $\beta$ -HBD,
    - c) a nitroprusside salt or a diazonium salt in a quantity sufficient to react with endogenous acetoacetate in the sample and acetoacetate obtained by conversion thereto of  $\beta$ -hydroxybutyrate in the sample,
    - d) a tetrazolium dye precursor ,
    - e) an electron mediator,
    - f) and a sufficient quantity of a buffer having a pH from about 8.6 to about 9.5 to maintain the strip at a level pH of about 8.6 to about 9.5 when saturated with sample.
15. A test strip according to Claim 14 wherein the  $\beta$ -HBD is from a source selected from among *Alcaligenes* and others capable of producing  $\beta$ -HBD that is uninhibited by chloride ions and is present in an amount of from about 0.2 to about 5.0 U per strip.

16. A test strip according to Claim 14 wherein the  $\beta$ -HBD is obtained from a source such that it is inhibited by chloride ions and is present in an amount per strip from about 1 to about 100 U per strip.
17. A test strip according to Claim 14 wherein the electron mediator is a diaphorase enzyme.
18. A test strip according to Claim 14 wherein the tetrazolium dye precursor is NBT or INT.
19. A test strip according to Claim 14 wherein ingredient (c) is sodium nitroprusside.
20. A test strip according to Claim 14 wherein ingredient (c) is a diazonium salt.
21. A test strip according to Claim 20 wherein ingredient (c) is 4-nitrobenzene diazonium fluoborate.
22. A test strip according to claim 2 comprising
  - 1) an inert support layer
  - 2) a dried reagent layer comprising a porous material impregnated with:
    - a) NAD
    - b)  $\beta$ -HBD
    - c) a nitroprusside salt or a diazonium salt in a quantity sufficient to react with endogenous acetoacetate in the sample and acetoacetate obtained by conversion thereto of  $\beta$ -hydroxybutyrate in the sample,

- d) and a sufficient quantity of a buffer having a pH from about 8.6 to about 9.5 to maintain the strip at a level of about 8.6 to about 9.5 when saturated with a sample from the group consisting of urine, saliva and sweat.
23. A test strip according to Claim 22 wherein the  $\beta$ -HBD is from a source selected from among *Alcaligenes* and others capable of producing  $\beta$ -HBD that is uninhibited by chloride ions and is present in an amount from about 0.2 to about 5.0 U per strip.
24. A test strip according to Claim 22 wherein the  $\beta$ -HBD is obtained from a source such that it is inhibited by chloride ions and is present in an amount per strip from about 1 to about 100 U per strip.
25. A test strip according to Claim 22 wherein the ingredient (c ) is a nitroprusside salt.
26. A test strip according to Claim 25 wherein ingredient ( c ) is sodium nitroprusside.
27. A test strip according to Claim 22 wherein ingredient ( c ) is a diazonium salt.
28. A test strip according to Claim 27 wherein ingredient ( c ) is 4 nitrobenzene diazonium fluoborate.

29. A test strip according to Claim 3 comprising
- 1) an inert support layer and
  - 2) a dried reagent layer comprising
    - a)  $\beta$ -HBD
    - b) NAD
    - c) nitroprusside salt or a diazonium salt in sufficient quantity to
      - (i) immediately react with the acetone present in the sample and stabilize it against volatilization
      - (ii) also react with the endogenous acetoacetate in the sample and with acetoacetate obtained by conversion thereto of  $\beta$ -hydroxybutyrate in the sample
    - d) a sufficient quantity of a buffer having a pH from about 8.6 up to about 9.5 to maintain the reaction pH between about 8.6 and about 9.5 when the strip is saturated with sample.
30. A test strip according to Claim 29 wherein the  $\beta$ -HBD is obtained from *Aliccaligenes* or another source such that it is not inhibited by chloride ions and it is present in an amount of about 0.2 to 5.0 U per strip.

31. A test strip according to Claim 29 wherein the  $\beta$ -HBD is obtained from a source such that it is inhibited by chloride ions and it is present in an amount from 1.0 to about 100 U per strip.
32. A test strip according to Claim 29 in which the salt is a nitroprusside salt.
33. A test strip according to Claim 32 in which the nitroprusside salt is sodium nitroprusside.
34. A test strip according to Claim 29 in which the salt is a diazonium salt.
35. A test strip according to Claim 34 in which the diazonium salt is 4-nitrobenzene diazonium fluoborate.
36. A method for monitoring the level of  $\beta$ -hydroxybutyrate present in a sample of human bodily fluid which comprises contacting a sample of said fluid with a mixture of
- a)  $\beta$ -HBD which has been obtained from a *Alcaligenes* or another source such that is uninhibited by chloride ions,
  - b) NAD,
  - c) a tetrazolium dye precursor,
  - d) an electron mediator capable of transferring an electron to said dye precursor to effect a color change and
  - e) a buffer having a pH of from about 8.6 to about 9.5,
- and measuring by electrochemical, spectrophotometric or fluorometric means, or by comparison of the color developed to a preestablished color intensity standard, the amount of  $\beta$ -hydroxybutyrate in the sample.



37. A method according to Claim 36 wherein the tetrazolium dye precursor is NBT or INT.
38. A method according to Claim 36 wherein the electron mediator is a diaphorase enzyme.
39. A method for monitoring the level of combined acetoacetate and  $\beta$ -hydroxybutyrate present in a sample of human bodily fluid which comprises contacting the sample with a mixture comprising the following ingredients:
- a)  $\beta$ -HBD which has been obtained from *Alcaligenes* or another source such that it is not inhibited by chloride ions,
  - b) NAD,
  - c) a tetrazolium dye precursor,
  - d) an electron mediator capable of transferring an electron to said dye precursor to effect a color change, and
  - e) a buffer having a pH from about 7.0 to about 8.3,
- and measuring by electrochemical, spectrophotometric or fluorometric means, or by comparison of the color developed to a preestablished color intensity standard, the amount of  $\beta$ -hydroxybutyrate plus acetoacetate present in the sample.
40. A method according to Claim 39 wherein the tetrazolium dye precursor is NBT or INT.
41. A method according to Claim 39 wherein the electron mediator is diaphorase enzyme.

42. A method for monitoring the level of combined  $\beta$  hydroxybutyrate and acetoacetate present in a sample of human bodily fluid which comprise contacting said sample with a mixture comprising the following ingredients:

- a)  $\beta$ -HBD which has been obtained from *Alcaligenes* or another source such that it is not inhibited by chloride ions,
- b) NAD,
- c) a nitroprusside salt of react with endogenous acetoacetate in the sample and acetoacetate obtained by conversion thereto of  $\beta$ -hydroxyrate in the sample, and
- d) a buffer having a pH of from about 8.6 to about 9.5

and measuring by electrochemical, spectrophotometric or fluorometric means, or by comparison of the color developed to a preestablished color intensity standard, the amount of combined acetoacetate and  $\beta$ -hydroxybutyrate in the sample.

43. A method according to Claim 42 wherein ingredient (c) is a nitroprusside salt.

44. A method according to Claim 43 in which the nitroprusside salt is sodium nitroprusside.

45. A method according to Claim 42 wherein ingredient (c) is a diazonium salt.

46. A method according to Claim 45 wherein the diazonium salt is 4-nitrobenzenediazonium.

47. A method according to Claim 42 having increased sensitivity wherein a tetrazolium dye precursor and an electron mediator are included in the mixture in addition to ingredients (a), (b), (c) and (d).
48. A method according to Claim 47 in which the tetrazolium dye precursor is NBT or INT and the electron mediator is a diaphorase enzyme.
49. A method for monitoring the level of total ketone bodies in a sample of human bodily fluid which comprises contacting said sample with a mixture containing the following ingredients:
- a)  $\beta$ -HBD which has been obtained from *Alcaligenes* or another source such that it is not inhibited by chloride ions,
  - b) NAD,
  - c) a nitroprusside or diazonium salt in a quantity sufficient to
    - (i) react instantaneously with and stabilize against volatilization the acetone in the sample,
    - (ii) react with endogenous acetoacetate in the sample and
    - (iii) react with acetoacetate formed by conversion thereto to  $\beta$ -hydroxybutyrate in the sample, and
  - d) a buffer having pH of from about 8.6 to about 9.5 and measuring by electrochemical, spectrophotometric or fluorometric means, or by comparison of the color developed to a preestablished color intensity standard, the amount of total ketone bodies in the sample.


50. A method according to Claim 49 wherein & ingredient ( c) is a nitroprusside salt.
51. A method according to Claim 50 wherein ingredient ( c) is sodium nitroprusside.
52. A method according to Claim 51 wherein ingredient ( c) is a diazonium salt.
53. A method according to Claim 52 wherein ingredient ( c) is 4-nitrobenzene diazonium fluoborate.
54. A method for monitoring the level of  $\beta$ -hydroxybutyrate present in a sample of human bodily fluid which comprises contacting a sample of said fluid with a mixture containing the following ingredients:
- a) at least 20 U per milliliter ("ml.") of  $\beta$ -HBD obtained from a source such that it is inhibited by chloride ions,
  - b) NAD,
  - c) a tetrazolium dye precursor,
  - d) an electron mediator capable of transferring an electron to said dye precursor to effect a color change and
  - e) a buffer having a pH of from about 8.6 to 9.5,

and measuring by electrochemical, spectrophotometric or fluorometric means or by comparison of the color developed to a preestablished color intensity standard, the amount of  $\beta$ -hydroxybutyrate in the sample.

55. A method according to Claim 54 wherein the tetrazolium dye precursor is NBT or INT.
56. A method according to Claim 54 wherein the electron mediator is a diaphorase enzyme.
57. A method for monitoring the level of combined acetoacetate and  $\beta$ -hydroxybutyrate present in a sample of human bodily fluid which comprises contacting the sample with a mixture comprising the following ingredients:
- a) at least 20 U per ml of  $\beta$ -HBD which has been obtained from a source such that it is inhibited by chloride ions,
  - b) NAD,
  - c) a tetrazolium dye precursor,
  - d) an electron mediator capable of transferring an electron to said dye precursor to effect a color change and
  - e) a buffer having a pH from about 7.0 to about 8.3,
- and measuring by electrochemical, spectrophotometric or fluorometric means, or by comparison of the color developed to a preestablished color intensity standard, the amount of acetoacetate plus  $\beta$ -hydroxybutyrate present in the sample.
58. A method according to claim 57 wherein the tetrazolium dye precursor is NBT or INT.
59. A method according to claim 57 wherein the electron mediator is diaphorase enzyme.

60. A method for monitoring the level of combined  $\beta$ -hydroxybutyrate and acetoacetate present in a sample of human bodily fluid which comprised contacting said sample with a mixture comprising the following ingredients:
- a) at least 20 U per ml. of  $\beta$ -HBD which has been obtained from a source such that it is inhibited by chloride ions,
  - b) NAD,
  - c) a nitroprusside salt or a diazonium salt in an amount sufficient to react with endogenous acetoacetate in the sample and acetoacetate obtained by conversion thereto of  $\beta$ -hydroxybutyrate in the sample, and
  - d) a buffer having a pH of from about 8.6 to about 9.5,
- and measuring by electrochemical, spectrophotometric or fluorometric means, or by comparison of the color developed to a preestablished color intensity standard, the amount of combined acetoacetate and  $\beta$ -hydroxybutyrate present in the sample.
61. A method according to Claim 60 wherein ingredient (c) is a nitroprusside salt.
62. A method according to Claim 61 wherein ingredient (c) is sodium nitroprusside.
63. A method according to Claim 60 wherein ingredient (c) is a diazonium salt.
64. A method according to Claim 63 wherein ingredients (c) is 4-nitrobenzene diazonium fluoborate.

65. A method according to Claim 60 having increased sensitivity wherein a tetrazolium dye precursor and an electron mediator are included in said mixture in addition to ingredients (a), (b), (c) and (d).
66. A method according to Claim 65 wherein the tetrazolium dye precursor is NBT or INT and the electron mediator is a diaphorase enzyme.
67. A method for monitoring the level of total ketone bodies present in a sample of human bodily fluid which comprises contacting said sample with a mixture containing
- a) at least 20 U per ml. of  $\beta$ -HBD which has been obtained from a source such that it is inhibited by chloride ion,
  - b) NAD,
  - c) a nitroprusside or a diazonium salt in a quantity sufficient to
    - (i) react instantaneously with and stabilize against volatilization the acetone in the sample,
    - (ii) react with endogenous acetoacetate in the sample and
    - (iii) react with acetoacetate formed by conversion thereto of  $\beta$  hydroxybutyrate in the sample, and
  - d) a buffer having a pH of from about 8.6 to about 9.5, and measuring by electrochemical, spectrophotometric or fluorometric means, or by comparison of the color developed to a preexisting color intensity standard, the amount of total ketone bodies in the sample.

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68. A method according to Claim 67 wherein ingredient ( c) is a nitroprusside slat.
  69. A method according to Claim 68 wherein ingredient ( c) is a sodium  
nitroprusside.
  70. A method according to Claim 67 wherein ingredient ( c) is a diazonium slat.
  71. A method according to Claim 70 wherein ingredient ( c) is 4-nitrobenzene  
fluoborate.